

**AMENDMENTS TO THE CLAIMS**

This listing of the claims will replace all prior versions, and listings, of claims in the application:

**LISTING OF CLAIMS:**

1 - 53. (cancelled)

54. (Currently amended) A process for producing a peptide having desired biological activity, comprising the steps of:

(1) culturing cells transformed with an expression vector having a nucleotide sequence encoding a fusion protein comprising:

(a) a protective peptide, and

(b) a peptide of interest connected to a helper peptide via cleavage site, wherein said protective peptide, said peptide of interest, and said helper peptide each have a different isoelectric point prior to use in said fusion protein;

and then harvesting said fusion protein from said culture, wherein said helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of the peptide of interest connected to a helper peptide is between 8 and 12, and further wherein there are cleavage sites between the protective peptide, the helper peptide, and the peptide of interest so that the fusion protein formed by said peptides contains two cleavage sites;

(2) cleaving off from said fusion protein said peptide of interest connected to a helper peptide via cleavage site, and purifying said peptide of interest connected to a helper peptide via cleavage site as desired;

(3) cleaving off from said peptide of interest connected to a helper peptide via cleavage site obtained in step (2), said peptide of interest; and

(4) purifying said peptide of interest obtained in step (3);

and wherein said peptide of interest is ~~SEQ ID NOS: 27-70~~ or a glucagon-like peptide 1 (GLP-1) derivative selected from the group consisting of:

(a) GLP-1(7-34), GLP-1(7-35), GLP-1(7-36), GLP-1(7-37), GLP-1(7-34)NH<sub>2</sub>, GLP-1(7-35)NH<sub>2</sub>, GLP-1(7-36)NH<sub>2</sub>, and GLP-1(7-37)NH<sub>2</sub>;

(b) GLP-1(7-37)-Arg, GLP-1(7-37)-Arg-Arg, GLP-1(7-37)-Lys, GLP-1(7-37)-Lys-Lys, GLP-1(7-37)-Lys-Arg, GLP-1(7-37)-Arg-Lys, and the C-terminal-amidated forms thereof;

(c) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 is substituted with Thr, Gly, or Ser;

(d) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg;

(e) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 34 of GLP-1 is substituted with Arg;

(f) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the arginine at position 36 of GLP-1 is substituted with Lys;

(g) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the lysine at position 26 is substituted with Arg;

(h) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the lysine at position 34 is substituted with Arg;

(i) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the arginine at position 36 is substituted with Lys;

(j) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, and the lysine at position 34 is substituted with Arg;

(k) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(l) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(m) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the lysine at position 34 is substituted with Arg;

(n) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(o) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(p) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys; and

(q) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys.

55. (previously presented) The process according to claim 54, wherein said protective peptide has 30 to 200 amino acid residues.

56. (previously presented) The process according to claim 54, wherein an ion exchange resin is used in the purification process.

57. (previously presented) The process according to claim 56, wherein said ion exchange resin is a cation exchange resin.

58. (previously presented) The process according to claim 54, wherein a reverse phase chromatography or a hydrophobic chromatography is used in the purification process.

59. (previously presented) The process according to claim 54, wherein a surfactant and/or a salt are added in at least one of steps (1) to (4) to maintain the solubility of said peptide of interest.

60. (previously presented) The process according to claim 54, wherein said cells are prokaryotic or eukaryotic cells.

61. (previously presented) The process according to claim 60, wherein said cells are *Escherichia coli* cells.

62. (previously presented) The process according to claim 54, wherein said peptide of interest is an amidated peptide.

Claim 63. Cancelled.

64. (previously presented) The process according to claim 63, wherein said peptide of interest has an isoelectric point of 4.5 to 9.0.

65. (previously presented) The process according to claim 63, wherein said peptide of interest has an isoelectric point of 5.5 to 7.5.

66. (previously presented) The process according to claim 63, wherein the purity of said glucagon-like peptide-1 derivative obtained having insulinotropic activity is 98% or greater.

67. (previously presented) The process according to claim 54, wherein said peptide of interest obtained in step (2) is subjected to a modification reaction to obtain a modified peptide.

68. (previously presented) The process according to claim 67, wherein said modification reaction is an amidation.

Claim 69. Cancelled.

70. (previously presented) The process according to claim 69, wherein said peptide of interest has an isoelectric point of 4.5 to 9.0.

71. (previously presented) The process according to claim 69, wherein said peptide of interest has an isoelectric point of 5.5 to 7.5.

72. (Currently amended) An expression vector comprising a nucleotide sequence encoding a fusion protein comprising a protective peptide and a peptide of interest having a helper peptide added thereto, wherein said protective peptide, said peptide of interest, and said helper peptide each have a different isoelectric point prior to use in said fusion protein, further wherein said helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of said peptide of interest that has the helper peptide added thereto is between 8 and 12, and further wherein there are cleavage sites between said protective peptide, said helper peptide, and said peptide of interest so that the fusion protein formed by said peptides contains two cleavage sites; and

wherein said peptide of interest is ~~SEQ ID NO: 27-70~~ or a glucagon-like peptide 1 (GLP-1) derivative selected from the group consisting of:

(a) GLP-1(7-34), GLP-1(7-35), GLP-1(7-36), GLP-1(7-37), GLP-1(7-34)NH<sub>2</sub>, GLP-1(7-35)NH<sub>2</sub>, GLP-1(7-36)NH<sub>2</sub>, and GLP-1(7-37)NH<sub>2</sub>;

(b) GLP-1(7-37)-Arg, GLP-1(7-37)-Arg-Arg, GLP-1(7-37)-Lys, GLP-1(7-37)-Lys-Lys, GLP-1(7-37)-Lys-Arg, GLP-1(7-37)-Arg-Lys, and the C-terminal-amidated forms thereof;

(c) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 is substituted with Thr, Gly, or Ser;

(d) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg;

(e) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 34 of GLP-1 is substituted with Arg;

(f) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the arginine at position 36 of GLP-1 is substituted with Lys;

(g) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the lysine at position 26 is substituted with Arg;

(h) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the lysine at position 34 is substituted with Arg;

(i) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, and the arginine at position 36 is substituted with Lys;

(j) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, and the lysine at position 34 is substituted with Arg;

(k) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(l) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(m) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the lysine at position 34 is substituted with Arg;

(n) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(o) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys;

(p) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the lysine at position 26 of GLP-1 is substituted with Arg, the lysine at position 34 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys; and

(q) GLP-1(7-37) and GLP-1(7-36)NH<sub>2</sub>, wherein the alanine at position 8 of GLP-1 is substituted with Thr, Gly or Ser, the lysine at position 26 of GLP-1 is substituted with Arg, and the arginine at position 36 is substituted with Lys.

73. (previously presented) A prokaryotic or a eukaryotic cell transformed with the expression vector of claim 72.

Claims 74-75. Cancelled.

76. (previously presented) An *Escherichia coli* cell transformed with the expression vector of claim 72.

Claim 77. Cancelled.

78. (previously presented) The process according to claim 54, wherein the order of said protective peptide, said peptide of interest, and said helper peptide contained within said fusion protein is, read from N-terminus to C-terminus: helper peptide, peptide of interest, protective peptide.

79. (currently amended) The process according to claim 54, wherein the order of said protective peptide, said peptide of interest, and said helper peptide contained within said fusion protein is[[,]] read from N-terminus to C-terminus: protective peptide, helper peptide, peptide of interest.

80. (previously presented) The process according to claim 54, wherein the order of said protective peptide, said peptide of interest, and said helper peptide contained within said fusion protein is, read from N-terminus to C-terminus: protective peptide, peptide of interest, helper peptide.

81. (previously presented) The process according to claim 54, wherein the order of said protective peptide, said peptide of interest, and said helper peptide contained within said fusion protein is, read from N-terminus to C-terminus: peptide of interest, helper peptide, protective peptide.

82. (previously presented) The process according to claim 68, wherein the fusion protein comprises the amino acid sequence shown in SEQ ID NO: 20.

83. (previously presented) The process according to claim 68, wherein the fusion protein consists of the amino acid sequence shown in SEQ ID NO: 20.

84. (withdrawn) The process according to claim 68, wherein the fusion protein comprises the amino acid sequence shown in SEQ ID NO: 21.

85. (withdrawn) The process according to claim 68, wherein the fusion protein consists of the amino acid sequence shown in SEQ ID NO: 21.

86. (withdrawn) The process according to claim 68, wherein the fusion protein comprises the amino acid sequence shown in SEQ ID NO: 22.

87. (withdrawn) The process according to claim 68, wherein the fusion protein consists of the amino acid sequence shown in SEQ ID NO: 22.

88. (withdrawn) The process according to claim 68, wherein the fusion protein comprises the amino acid sequence shown in SEQ ID NO: 23.

89. (withdrawn) The process according to claim 68, wherein the fusion protein consists of the amino acid sequence shown in SEQ ID NO: 23.

90. (withdrawn) The process according to claim 54 wherein the helper peptide comprises SEQ ID NO: 5.

91. (withdrawn) The process according to claim 54, wherein the helper peptide comprises SEQ ID NO: 8.

92. (withdrawn) The process according to claim 69, wherein the peptide of interest consists of the amino acid sequence shown in SEQ ID NO: 27.



93. (previously presented) The process according to claim 54, wherein the protective peptide consists of amino acid numbers 1-98 of SEQ ID NO: 20.

94. (previously presented) The process according to Claim 54, wherein the peptide of interest is selected from the group consisting of the peptides of SEQ ID NOS: 27 to 70.

95. (previously presented) The process according to Claim 54, wherein the peptide of interest is selected from the group consisting of the peptides of SEQ ID NOS: 27 and 28.

96. (previously presented) The process according to Claim 54, wherein the peptide of interest comprises the peptide of SEQ ID NO: 28.

97. (previously presented) A process for producing a peptide having desired biological activity, comprising the steps of:

(1) culturing cells transformed with an expression vector having a nucleotide sequence encoding a fusion protein comprising:

(a) a protective peptide, and

(b) a peptide of interest connected to a helper peptide via cleavage site, wherein said protective peptide, said peptide of interest, and said helper

peptide each have a different isoelectric point prior to use in said fusion protein;

and then harvesting said fusion protein from said culture, wherein said helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of the peptide of interest connected to a helper peptide is between 8 and 12, and further wherein there are cleavage sites between the protective peptide, the helper peptide, and the peptide of interest so that the fusion protein formed by said peptides contains two cleavage sites;

(2) cleaving off from said fusion protein said peptide of interest connected to a helper peptide via cleavage site, and purifying said peptide of interest connected to a helper peptide via cleavage site as desired;

(3) cleaving off from said peptide of interest connected to a helper peptide via cleavage site obtained in step (2), said peptide of interest; and

(4) purifying said peptide of interest obtained in step (3)  
wherein the fusion protein is a protein selected from the group consisting of SEQ ID NOS: 20, 21, 22 and 23.

98. (previously presented) The process according to claim 68, wherein the peptide of interest is GLP-1(7-36)NH<sub>2</sub>.